

THE EXTRACTION OF ENCHYTRAEIDAE FROM SOIL

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Details are given of the Nielsen-extractor and a modified Baermann-funnel method. In the first, the soil is heated in a vessel immersed in a water-bath and the worms are driven upwards into a cooled layer of fine gravel resting on top of the sample. The worms are recovered from the sand by washing and decantation. In the second, heat is applied to the soil on a sieve resting in a funnel full of water. The worms move downwards into the stem of the funnel from which they can be collected. The two methods appear to give comparable results when used by their respective authors but the funnel technique is simpler and probably less influenced by variations in design. While the percentage efficiency of these methods cannot be estimated, it has been shown that both give a constant extraction efficiency and reproduce the characteristic features of distributions of Enchytraeidae with a high degree of accuracy.

UNTIL recently no adequate technique for the assessment of enchytraeid population densities existed, though JEGEN (1920) and MOSZYŃSKI (1930-3) had recorded high numbers from certain soils. These workers obtained the worms by hand-sorting the soil, either dry or in a tray of water. Moszyński recommended the addition of a few drops of alcohol to the water in order to activate the worms and make them more readily detectable. Hand-sorting is not only time consuming but inaccurate and cannot be used for population-census studies. Recently two techniques for the large-scale extraction of Enchytraeidae from soil have been described by NIELSEN (1952-3) and O'CONNOR (1955).

THE NIELSEN EXTRACTOR

This technique (NIELSEN, 1952-3) was first developed for use in a poor, permanent pasture on an acid, sandy soil. The soil is heated in an earthenware vessel immersed in a water-bath and the worms are driven upwards into a cooled layer of sand or fine gravel resting on top of the soil sample (*Figure 87*). The glazed, earthenware cylinder (*A*) is 14.5 cm high and 7.5 cm diameter (internal dimensions), and has a hole (*ca.* 7 mm *d*) in its base. The bottom of the vessel is filled to a depth of 3 cm with gravel (*B*), which is covered with wire gauze (*E*, 1 mm mesh) fastened to the wall of the vessel with plaster of Paris (*F*). The apparatus is designed to take soil cores 5 cm deep and 7.3 cm in diameter obtained with a stainless-steel cylindrical sampling tool; deeper cores must be subdivided into 5-cm layers. The soil core is obtained

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in an undisturbed condition and, in the field, is placed with upper surface upwards in the extraction vessel so that it rests on the ring of plaster of Paris (*F*); this ensures that there is a small air space between it and the wire gauze. The soil (*G*) is covered with a layer of fine gravel (*H*, 2.5 cm deep) having a particle size of 2–4 mm; the gravel must be carefully washed to remove organic debris and clay particles. A cooling coil (*I*, *ca.* 4 mm internal diameter) connected to the cold-water supply is embedded at a depth of 0.5 cm below the surface of the upper gravel layer and is supported by two wires (*J*), arranged at right angles with their ends hooked over the lip of the vessel.

The extraction vessel is immersed in a water-bath (*D*) so that the water comes to the upper surface of the gravel layer (*C*), and with the upper 6 cm

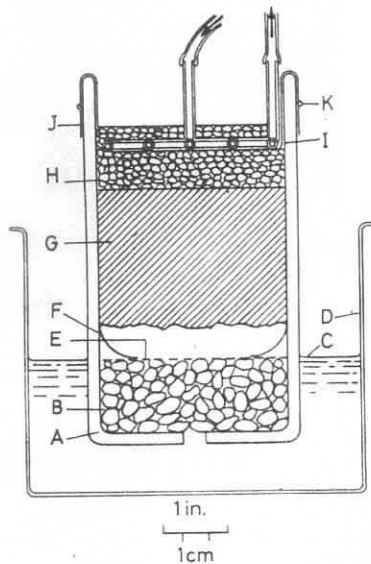


Figure 87. Nielsen extractor unit in section (after NIELSEN, 1952–3).

A, earthenware extraction vessel with 3-cm layer of gravel (*B*) in its base; *C*, water level of water-bath (*D*); *E*, wire gauze held in position with plaster of Paris (*F*); *G*, soil sample; *H*, fine-gravel layer, 2.5 cm deep; *I*, cooling coil with one of the two supporting wires (*J*) hooked over the rim of the vessel and secured by a rubber band (*K*).

of the vessel protruding above the rim of the bath. The bath is heated at a rate to provide, after about two hours, a water temperature of 60°C. At the same time water evaporates from the lower gravel surface and recondenses in the soil and thus a moisture gradient is established, high at the base and low at the top of the sample. When the upper 1 cm of the soil core reaches 25°C (after *ca.* 1 hour) cold water is circulated through the cooling coil resulting in an abrupt decrease in temperature in this layer (*Table 47*). Heating is continued until the water reaches 85°C and the upper 1 cm of the sample about 35°C. This temperature level is maintained for about one hour, when the upper gravel layer is removed and the worms recovered by repeated washing and decantation.

The complete process takes three hours and a number of samples may be extracted simultaneously in a single water-bath. In practice it is best to divide the cooling coils into sets of three and connect the members of each set in series, the inlets for each set being connected to a rake-like manifold.

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Table 47. Temperature gradient in Nielsen extractor after two hours' operation (in part after NIELSEN, 1952-3)

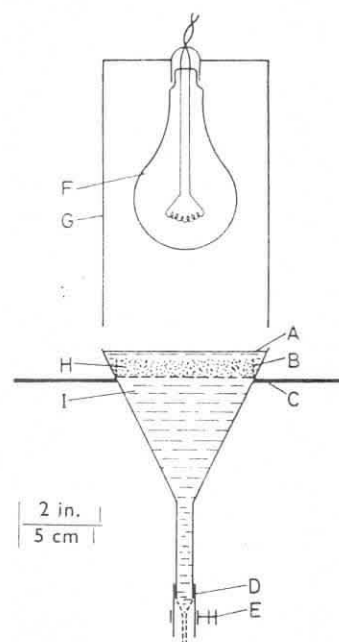
Material	Depth from surface of upper gravel layer (cm)	Temperature (°C)	
		No water circulating	Water circulating
Upper gravel layer—surface	0.5	26	16
—base	2.0	31	20
Soil core—near surface	4.0	42	42
—near base	6.0	49	49
Lower gravel layer	9.0	59	59
Water-bath	—	60	60

MODIFIED BAERMANN-FUNNEL METHOD

In this wet-funnel method (O'CONNOR, 1955)—a modification of one devised by BAERMANN (1917)—heat is applied to soil spread on a sieve resting in a funnel full of water (Figure 88). The worms move downwards through the sieve into the funnel from which they can be collected by releasing the

Figure 88. Modified Baermann-funnel extractor unit in section.

A, polythene funnel containing wire-gauze sieve (B), and supported by asbestos board (C); D, rubber tube with spring clip (E) attached to funnel outlet; (F) electric-light bulb enclosed in a metal cylinder (G); H, soil subcore submerged in water (I).



spring clip. This technique was first used in a study of the enchytraeid population of a pure stand of Douglas fir (*Pseudotsuga taxifolia* (Lamb)) in North Wales (O'CONNOR, 1957, 1957a). It has since proved suitable for a wide variety of woodland soils, peats, vegetable debris and agricultural land.

The apparatus consists of a polythene funnel (Figure 88, A; d 11 cm, 60°

included angle) in which rests a wire-gauze sieve (*B*, *d* 9 cm, 30 mesh). The funnels are inserted in holes (*d* 9 cm) in an asbestos board (*C*), and have a rubber tube (*D*) with spring clip (*E*) attached to the outlet. Above each funnel is suspended an electric-light bulb (*F*, 60 W) enclosed in a metal cylinder* (*G*, *d* 11 cm, *h* 18 cm). The funnels are arranged in batteries of twelve supported on a metal-alloy framework. The light bulbs and their cylinders are attached to a removable frame and wired in parallel. Heating is controlled either by a rheostat (*Table 48*) or by gradually lowering the frame from a height of about 10 cm.

The samples (*d* 6.3 cm and 6 cm deep) are obtained with a cylindrical sampler split longitudinally so that it can be opened and the exposed core

Table 48. Temperature changes during extraction with modified Baermann funnel and rheostat values for each stage

Time from commencement of heating (min)	Rheostat value (Ω)	Soil temperature ($^{\circ}\text{C}$)		
		Surface	Middle	Base
15	35.0	17.5	17.5	17.5
30	31.5	24.0	21.0	19.5
45	28.0	27.5	23.5	21.5
60	24.5	30.0	25.5	23.0
75	21.0	31.5	28.0	25.0
90	17.5	33.0	29.0	26.5
105	14.0	34.0	30.5	27.0
120	11.5	35.0	31.5	28.0
135	8.0	36.5	32.5	29.0
150	4.5	38.0	33.5	30.0
165	0.0	40.0	34.5	31.0
180		42.0	36.0	32.0
		44.0	37.5	33.5

subdivided into the natural divisions of the profile before removal from the sampler (O'CONNOR, 1957a); this minimizes the risk of damage to organisms by compression. Each soil horizon is further subdivided into 2-cm layers, and each subcore (*H*, *V* 187 cm³) is placed surface downwards on the sieve plate in the funnel. Disturbance of the soil during loading of the funnel has no effect on extraction efficiency. The funnel is then filled with water (*I*) so that the soil sample is completely submerged. The worms, which collect in the funnel stem, are withdrawn by releasing the spring clip. The heating of the funnel is increased gradually in steps as indicated in *Table 48*, and when the sample surface has reached a temperature of 45°C—after about 180 minutes—extraction is completed.

When the samples are dry, they must be moistened with a fine jet of water before immersion in the funnels. If the soil temperature is low they must be gradually equilibrated to room temperature over a 24-hour period prior to extraction.

* 'National Dried-milk' tins proved ideal for this purpose.

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COMPARISON OF NIELSEN EXTRACTOR AND MODIFIED BAERMANN FUNNEL

A comparison of the two methods (*Table 49*) by their respective authors using samples from the same site—a permanent pasture on sandy soil—showed no significant difference ($P > 0.9$) in extraction efficiency*. However, in a comparison of the two methods using the coniferous-forest soil (O'CONNOR, 1955) already referred to, the difference between means

Table 49. Numbers of Enchytraeidae extracted by Nielsen extractor and wet funnel from a permanent pasture on sandy soil (sampling unit 36 cm² × 5 cm deep)

Method	Number per sampling unit													Mean number and standard error
Nielsen extractor	137	132	128	123	113	109	108	105	98	60	47	15	97.9 ± 36.1	
Wet funnel	156	130	130	119	116	114	114	107	106	93	77	75	111.4 ± 21.7	

$$t = 1.063; P > 0.9$$

(*Table 50*) was highly significant ($P < 0.01$), and indicated a superiority for the wet-funnel method. NIELSEN (1952-3), in his experiments, also refers to a partial failure of the wet-funnel method. From this evidence it seems that the personal factor whereby an extraction technique is likely to be most successful when used by its designer must be considered important. The practical implication of this is that the original description of a technique should be carefully followed. Experience has shown that even minor deviations from the original design may result in a considerable loss in

Table 50. Numbers of Enchytraeidae extracted by Nielsen extractor and wet funnel from a coniferous forest soil (sampling unit 46.3 cm × 6 cm deep)

Method	Number per sampling unit												Mean number and standard error
Nielsen extractor	258	164	149	137	81	63	57	57	56	54			107.8 ± 64.7
Wet funnel	654	641	339	320	277	240	216	163	161	137			313.9 ± 176.4

$$t = 3.29; P < 0.01$$

efficiency. For example the use of material that is not sufficiently coarse in the upper gravel layer of the Nielsen extractor will have a serious effect, and any attempt to increase the amount of soil per unit in the wet funnel will be equally liable to failure. In both methods the rate of heating is the most important single factor influencing extraction efficiency.

* For a comparative test of the two methods with moorland soil, see Peachey, p. 287—Ed.

Both techniques, relying on the movement of the worms in response to stimuli, cannot recover aestivating worms or cocoons. Methods of extraction involving the mechanical dispersion of soil particles are unsuitable because they cause severe damage to the worms.

The wet-funnel technique has the advantage that the apparatus is simple and easy to operate; heating is easily controlled and extraction is a single process. This may render it less liable to failure in the hands of other workers. However, soil type, soil-moisture content, surface vegetation and the species composition of the population may give either method an advantage in a particular habitat. Where it is necessary to use larger samples the Nielsen extractor will be probably more easily adapted as an increase in the diameter of the extraction cylinders will not affect the form of the temperature gradient. The wet funnel cannot be used for larger sizes without modification of the heating method because horizontal temperature gradients tend to occur when the diameter of the funnel is increased. However, the latter method is readily applicable to smaller core sizes either by increasing the thickness of the subcores or by decreasing the diameter of the extractor units. Any alteration in the dimensions of either apparatus will necessitate a complete recalibration.

There is no valid test of extraction efficiency for a technique relying on the movement of organisms in response to stimuli. However, with the methods described here it is possible to demonstrate a constant degree of efficiency. Part of the evidence for the success of these techniques, and for their comparability when used by their respective authors, lies in the results obtained from complete counts of the populations in small areas. Maps of the spatial distribution of individuals in the population based on such data are characterized by a pattern of interrelated aggregations and distinct density gradients are detectable from aggregation centres (NIELSEN, 1954; O'CONNOR, 1957). The fact that continuous contours delimiting areas of given relative density can be detected, proves that extraction efficiency cannot be erratic, and the similarity of maps prepared from data obtained using the two methods points to their comparability. When sampling is more widely dispersed the two methods give an identical distribution of observations around sample means, and when the standard deviations are plotted against mean numbers per soil core, the slope of the line obtained is identical for each method (NIELSEN, 1955; O'CONNOR, 1957a). The similarity of the data obtained using the two methods again points to their comparability. Furthermore, the slope of the graphs relating standard deviation with mean density would be obscured by erratic extraction efficiency. Although this evidence does not indicate absolute extraction efficiency, it is apparent that both methods give an accurate reproduction of the characteristic features of the distribution of individuals in the population.

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